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银桦酸和银桦内酯的生源探讨*

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新的植物产物银桦内酯（Ⅰ）(Grevillone)（即6-羟基香豆素，6-Hydroxycoumarin)与其结构相应的开环酸——银桦酸(Ⅱ) (Grevillic acid)（即2，5-二羟基-反式-桂皮酸，2，5-Dihydroxy-*trans*-cinnamic acid）共存于山龙眼科（*Proteaceae*）的银桦（*Grevillea robusta* A. Cunn.）叶[1]，提示它们在生物化学上是密切相关的[2]。并且，无论是银桦内酯或银桦酸，在植物界里迄今仅发现于银桦[1]中。

桂皮酸(Ⅲ) (Cinnamic acid)的羟基化衍生物及其结构相应的内酯——香豆素类化合物，都是高等植物的典型代谢产物[4]。在双子叶植物里，苯基丙氨酸(Phenylalanine)是它们的共同前体[4,5]。最常见的羟基桂皮酸类是*p*-香豆酸（Ⅳ）（*p*-Coumaric acid)，咖啡酸(Ⅴ) (Caffeic acid)，阿魏酸(Ⅵ) (Ferulic acid)和芥子酸(Ⅶ) (Sinapic acid)。它们之中的一个或几个，以酯或甙的结合形式存在于每种高等植物[6]中，并且在代谢上密切相关[3,4]。这几个酚酸的共同特征是在对位碳上连接着氧原子（羟基）。显然，这与几乎所有天然香豆素类（Coumarins）化合物都在7位碳（C_7）上连接着氧原子[7,8]的特征在结构关系上是一致的。表1列出了在高等植物里最常见的几个羟基桂皮酸[2,4]和香豆素类化合物[4,7]。邻-羟基桂皮酸类[6]（*o*-Hydroxycinnamic acids）和间-羟基桂皮酸（*m*-Hydroxycinnamic acid）在植物界里较罕见。

对-羟基桂皮酸类、邻-羟基桂皮酸和C_7上连接氧原子的香豆素类化合物以及香豆素本身的生物合成途径机制（Mechanism for Biosynthetic Pathways）已有很多研究[2,4,9,20]。在双子叶植物里，都是以混合功能氧化酶(Mixed Function Oxygenase)催化羟基化反式-桂皮酸，发生NIH-位移[4,10] (National Institute of Health-shift)，构成酚性反式-桂皮酸衍生物（Phenolic-*trans*-cinnamic acid derivatives）。继葡萄糖化（Glucosylation）后，重排为相应的顺式-桂皮酸类葡糖甙（*cis*-cinnamic acids glucoside），经邻位脱糖即环化为相应的香豆素类化合物。图1表示从反式-桂皮酸转变为4-羟基-反式-桂皮酸（Ⅳ）和相继转变为伞形花内酯（Umbelliferone）的生物合成途经。

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I

银桦内酯

(Grevillone)

II

银 桦 酸

(Grevillic acid)

表1 高等植物里最常见的几个桂皮酸衍生物和香豆素类化合物

Table 1. The common hydroxycinnamic acids and coumarins in higher plant

桂皮酸衍生物	取 代 基			香豆素类
名 称	R	R′	R″	名 称
桂 皮 酸 (Ⅲ) (Cinnamic acid)	H	H	H	香 豆 素 (Coumarin)
p-香 豆 酸 (Ⅳ) (*p*-Coumaric acid)	OH	H	H	伞 形 花 内 酯 (Umbelliferone)
咖 啡 酸 (Ⅴ) (Caffeic acid)	OH	OH	H	七 叶 树 内 酯 (Aesculetin)
咖 啡 酸 (Ⅴ) (Caffeic acid)	OH	H	OH	瑞 香 内 酯 (Daphnetin)
阿 魏 酸 (Ⅵ) (Ferulic acid)	OH	OCH_3	H	东 莨 菪 内 酯 (Scopoletin)
芥 子 酸 (Ⅶ) (Sinapic acid)	OH	OCH_3	OCH_3	异 白 蜡 树 内 酯 (Isofraxetin)

混合功能氧化酶

反式-桂皮酸

trans-Cinnamic acid

环氧衍生物

Epoxy derivative

中 间 产 物 A

Intetmediate A

中 间 产 物 B

Intermediate B

NIH—位移 → HO—⟨苯环⟩—C(H)=CHCOOH —邻羟基化→ HO—⟨苯环(OH)⟩—C(H)=CHCOOH

4-羟基-反式-桂皮酸
4-Hydroxy-*trans*-cinnamic acid

2,4-二羟基-反式-桂皮酸
2,4-Dihydroxy-*trans*-cinnamic acid

葡萄糖化 → Glu.O—⟨苯环(OGlu.)⟩—C(H)=CHCOOH —重排→ Glu.O—⟨苯环(OGlu.)⟩—CH=CHCOOH

2,4-二羟基-反式-桂皮酸二葡糖甙
2,4-Dihydroxy-*trans*-cinnamic acid diglucoside

2,4-二羟基-顺式-桂皮酸二葡糖甙
2,4-Dihydroxy-*cis*-cinnamic acid diglucoside

脱糖 → Glu—O—⟨苯环(HO)⟩—CH=CHCOOH —环　化→ Glu.O—⟨香豆素环⟩(O, O)

2,4-二羟基-顺式-桂皮酸-4-葡糖甙
2,4-Dihydroxy-*cis*-cinnamic acid-4-glucoside

伞形花内酯葡糖甙
Umbelliferone glucoside
(Skimmin)

脱糖 → HO—⟨香豆素环⟩(O, O)

7-羟基香豆素（伞形花内酯）
7-Hydroxycoumarin (Umbelliferone)

图 1　4-羟基-反式-桂皮酸和 7-羟基香豆素的生物合成途径
Fig. 1. Biosynthetic-pathway for p-coumaric acid and umbelliferone

就生源观点而论，发现银桦叶能合成并能较丰富地积累银桦酸和银桦内酯这一现象是很有趣的。因为，以桂皮酸为前体（Precursor）转变为羟基桂皮酸衍生物和香豆素化合物的现有生物合成机制不能解释银桦酸和银桦内酯在植物体内的形成。而且，如表 2 所见，存在于银桦叶里的羟基桂皮酸的酯类[11,12,13] 与植物中通常发现的羟基桂皮酸的酯类[5] 也有某些差异。

表2　银桦与其它植物里发现的羟基桂皮酸的酯类

Table 2. Esters of hydroxy-cinnamic acids occur in G. robusta and othr plants

在植物叶里通常发现的桂皮酸衍生物的酯类	在银桦叶里发现的桂皮酸衍生物的酯类
氯原酸（Ⅷ） (Chlorogenic acid)	桂皮酰葡糖酯（Ⅸ） (Cinnamoylglucose)
p-苦马酰喹啉酸 (*p*-Coumaroylquinic acid)	R＝H 或 OH
4-羟基桂皮酰葡糖酯 (4-Hydroxycinnamoylglucose)	4-羟基桂皮酸甲酯（Ⅹ） (Methyl 4-hydroxycinnamate)

示踪原子试验表明[14]，在一般植物里，桂皮酸容易在对位、邻位或邻-对位羟基化。摸拟试验证明[15]，以过氧化酶（Peroxygenase）羟基化桂皮酸时，其产物几乎完全为邻-羟基桂皮酸和对-羟基桂皮酸，仅生成微量的2,5-二羟基桂皮酸。并且，银桦叶不含氯原酸[11]（Ⅷ），仅合成桂皮酸类的葡糖酯[12,3]（Ⅸ）（Glucose esters of the cinnamic acid）。对于一般植物，倘若将4-羟基桂皮酸甲酯（Ⅹ）（Methyl 4—hydroxycinnamate）施入叶内，其主要产物为4-羟基桂皮酰葡糖酯[16]（4-Hydroxycinnamoylglucose），而银桦叶能积累丰富的4-羟基桂皮酸甲酯[13]。报道指出[13,17]，在山龙眼科（*Proteaceae*）植物里，其前体可能是4-羟基苯甲酰乙酸甲酯（Methyl-(4-hydroxybenzoyl) acetate）。除银桦内酯外，在山龙眼科植物里，尚未发现香豆素类化合物[18,19]以及2,3-二羟基桂皮酸。在植物体内，以桂皮酸或水杨酸（Salicylic acid）作为前体，经混合功能氧化酶催化羟基化，能够产生2,5-二羟基苯甲酸[3,4]（2,5-Dihydroxybenzoic acid，即龙胆酸 Gentisic acid）。然而，龙胆酸在高等植物里分布非常广泛[6]。

因此，我们认为，在银桦叶里，桂皮酸被双氧化酶（Dioxygenase）催化羟基化为银桦酸（Grevillic acid，即2,5-二羟基-反式-桂皮酸），然后再转变为银桦内酯（Grevillone，即6-羟基香豆素）。其可能的反应机制如图2所示。首先由双氧化酶活化的一个氧分子进攻桂皮酸（Ⅲ）苯环的C_2或C_5，同时发生电子转移，经过不稳定的中间化合物（Ⅱi）（Intermediate），相继环化，构成环式过氧化物（Ⅱc）（Cyclic

$E-Fe^{3+}$

C=CHCOOH　　C=CH—COOH　　$+E-Fe^{+2}$

Ⅲ　　Ⅱi

C=CHCOOH　　C=CHCOOH

Ⅱc　　Ⅱg

C=CHCOOH　　C=CHCOOH

Ⅱ　　Ⅱg

CH=CHCOOH　　CH=CHCOOH

Ⅱcis　　Ⅱ5g

Ig　　I

图2　在银桦里由桂皮酸转变为银桦酸和银桦内脂的可能生物合成途径。

Fig. 2. Possible biosynthetic pathway for grevillic acid and grevillone

peroxide)。此环式过氧化物经分子内部氧化-还原作用（Intramolecular oxidation-reduction），转变为醌醇式化合物（Ⅱq）（Quinole）。醌醇式化合物（Ⅱq）经分子内重排（Intramolecular rearrangement）便生成银桦酸（Ⅱ）（Grevillic acid）。

从上述反应机制，可以看到，在桂皮酸由双氧化酶催化羟基化转变为银桦酸的历程中，并不发生 NIH-位移，而发生另一种类型的位移，即桂皮酸里互为对位的两个氢原子，在二羟基化过程中，转移到其各自对位的氧原子上，也就是说，原来C_2上的H转移为C_5-OH上的H，原来C_5上的H转移为C_2-OH上的H，对于这类位移，我们暂且称之为KIB-位移（Kunming Institute of Botany-shift）。

由银桦酸（Ⅱ）转变为银桦内脂（Ⅰ）的机制，则如其他取代反式桂皮酸衍生物转变为相应的香豆素类化合物[2,4]那样。即2,5-二羟基-反式-桂皮酸（Ⅱ）经葡糖化构成2,5-二羟基-反式-桂皮酸二葡糖甙（Ⅱg）（2,5-Dihydroxy-*trans*-cinnamic acid diglucoside），继以赖光反应（Light-dependent reaction）重排为2,5-二羟基-顺式-桂皮酸二葡糖甙（Ⅱcis）(2,5-Dihydroxy-*cis*-cinnamic acid diglucoside)，经酶脱糖，转变为2,5-二羟基-顺式-桂皮酸-5-葡糖甙（Ⅱ5g）（2,5-Dihydroxy-*cis*-cinnamic acid-5-glucoside），（Ⅱ5g）便自然地环化为银桦内脂葡糖甙（Ⅰg）（Grevillone glucoside)，再经脱糖，便生成银桦内脂（Ⅰ）(Grevillone)。

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A STUDY ON THE BIOGENESIS OF GREVILLIC ACID AND GREVILLONE

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Both grevillic (2,5-dihydroxy-*trans*-cinnamic) acid and grevillone (6-hydroxycoumarin) were only found from *Grevillea robusta* A. Cunn. (*Proteaceae*) thus far in the plant kingdom. It is of interest from biogenetic viewpoint that their hydroxylation pattern shows to be quite special. Basically, the problem of the biogenesis of grevillic acid and grevillone is this: by what enzymic pathway is this special hydroxylation pattern formed from *trans*—cinnamic acid in the plant?

In the present paper, an attempt is made to suggest the mechanism of the possible biosynthetic pathway for conversion of *trans*-cinnamic acid via grevillic acid to grevillone in the plant. Our suggestion is based on evidence of an indirect and structural character, and not purely speculative.

The mechanism for biosynthesis of the usual hydroxycinnamic acids and coumarins from *trans*-cinnamic acid which is hydroxylated by mean of catalysis of mixed function oxygenase, cannot satisfactorily explain to form for the infrequent grevillic acid and grevillone in the plant. Moreover, in view of what follows:

1. The tracer experiments on the biosynthesis of hydroxy-cinnamic acids had established that cinnamic acid fed to numerous plants is readily hydroxylated in para or ortho position or both para and ortho positions.

2. Model experiments with a peroxidase system had also shown that cinnamic acid is hydroxylated almost each and all in position para and ortho and only produced a trace of 2,5-hydroxycinnamic (grevillic) acid.

3. The common hydroxycinnamic acids are metabolically interrelated in the following way: cinnamic→*p*-coumaric→caffeic→ferulic→sinapic acids. These hydroxylations and methylations could occur through derivatives of acid, such as the quinolyl esters. Thus *trans*-(^{14}C) cinnamic acid is converted to cinnamoylquinic, *p*-coumaroylquinic and coffeoylquinic acids, cinnamoylglucose and

p—coumaroylglucose in the certain plants, but in *Grevillea robusta*, a species which does not contain coffeoylquinic (chlorogenic) acid, only glucose esters of the cinnamic acids were synthesized.

4. 1-*p*-Coumaroyl-β-D-glucose was the main product of feeding the leaves of a variety of plants with methyl *p*—coumarate. But a large amount of methyl *p*-coumarate may be accumulated in the leaves of G. *robusta*. Moreover, it was interesting to note that methyl (4-hydroxybenzoyl) acetate may be a precursor of methyl *p*-coumarate in the proteaceous plants.

5. Coumarins except grevillone, and 2, 3-dihydroxycinnamic acid have not yet been found in proteaceous family.

6. Gentisic (2, 5-hydroxybenzoic) acid which had an unusual hydroxylation pattern, is also produced from cinnamic and salicylic acids when the both are administered to plant, and the facility with which salicylic acid was hydroxylated in vivo to gentisic acid by mean of mixed function oxygenase. Moreover, gentisic acid is quite widely distributed in the plant kingdom.

In accordance with the above reasons, we have bring forward that grevillic acid is *Grevillea robusta* from *trans*-cinnamic acid which may be catalysed hydroxylation by mean of dioxygenase (Fig. 2). It probably begins with attack by an oxygen molecule activated by a dioxygenase at cardon atom 2 or carbon atom 5 of cinnamic acid (Ⅱ), and the same time, it is stirred a shift of electrons to form intermediate (Ⅱi). It followed a cyclization of carbon-oxygen atoms to form cyclic peroxide (Ⅱc) and made an intramolecular oxidation-reduction to form quinole (Ⅱq). Grevillic acid (Ⅰ) may be arise from this by an intramolecular rearrangement.

It might be seen that it cannot stir NIH-shift during the hydroxylation of cinnamic acid by mean of dioxygenase. However, it may appear an other type of shift. Namely, during the hydroxylation, both hydrogen atoms at the para position each other are shifted up to the oxygen atoms of the para position each and every. That is to say, hydrogen atom at the C-5 is shifted up to the OH at the C-2, hydrogen atom at the C-2 is shifted up to the OH at the C-5. This type of shift is for the first time being termed the "KIB-shift" (Kunming Institute of Botany-shift)

Grevillone is formed from grevillic acid, just as that most substituted coumarins are formed from the corresponding substituted *trans*-cinnamic acid derivatives,